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REVIEW

Primary Structure and Function of a Dynein Motor Molecule

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INTRODUCTION

Gibbons and Rowe [1] were the first to describe the microtubule-associated motor protein known as dynein, which they found when they isolated an ATPase from *Tetrahymena* cilia. The axonemal dyneins are contained in the outer and inner arms that project from the peripheral doublet microtubules, and they form cross bridges between adjacent doublet microtubules. The ATP-driven cross-bridge cycles generate the sliding between microtubules that gives rise to both flagellar and ciliary movements [2]. The outer and inner arm dyneins are different in terms of their peptide compositions. Both the dyneins are multimeric proteins and are composed of heavy chains with ATPase activity, which are assumed to be motor peptides, and several accessory peptides. The molecular mass of the heavy chains in the dyneins (~500 kDa) is 2.5-fold larger than that of the myosin heavy chain and 4.5-fold larger than that of the kinesin heavy chain, the other well-known motor molecules. Progress in analyzing the structure and function of dynein heavy chains was hindered by the lack of primary sequence information, which was due to the difficulties encountered in attempts to clone a cDNA that encodes such a large peptide. Nevertheless, the sequence was avidly pursued by many groups for quite sometime. Finally, Gibbons *et al.* [3] and Ogawa [4] simultaneously determined the complete amino-acid sequence of a dynein motor molecule: the well-characterized β heavy chain of sea-urchin axonemal dynein. The present review will be limited to a description of the "brave new world" of the sea-urchin axonemal dynein motor molecule, as revealed by molecular cloning.

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THE DYNEIN MOTOR MOLECULE

When demembranated sea-urchin sperm are extracted with high salt, the flagellar beat frequency of extracted sperm is only about half that of control sperm that have not been exposed to high salt [5]. Examination by electron microscopy revealed that extraction with high salt removes most of the outer arms from the doublet microtubules, leaving the inner arms apparently intact. The outer arms can be purified as ATPase-containing particles with as S value of 21 (referred to an the outer-arm dynein or 21S dynein) by centrifugation through a sucrose density gradient. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) resolved the outer-arm dynein into at least nine different peptides: α and β heavy chains (DαHC and DβHC); three intermediate chains (IC1-3); and at least four light chains (LCs) [6]. Exposure to a low-salt medium converts 21S dynein into three smaller factions: one containing the DβHC/IC1 complex; one containing aggregates of DαHC; and one containing IC2 and IC3 [7].

Sale *et al.* [8] were able to examine isolated outer-arm dynein by the quick-freeze, deep-etch technique. Replicas revealed that the 21S particles were composed of two globular heads jointed by two irregularly shaped stems that made contact along their length. One head was pear-shaped and the other was spherical. The stems were decorated

with a complex of bead-like particles. The Dβ HC/IC1 complex, obtained as described above, contained only single-headed molecules with single stems. These heads were predominantly pear-shaped. Sale *et al.* concluded that each head is formed by a heavy chain, that the pear-shaped head contains the Dβ HC, and that the spherical head contains the Dα HC. Three intermediate chains might decorate the stem that is joined to each head. The position *in situ* of LCs in the outer arm has not been described. Sale *et al.* also observed *in situ* the outer-arm dynein of demembranated sptem. When frozen in reactivation buffer in the absence of ATP, each arm consists of a large globular head that is attached to the A-subfibers of doublet microtubules *via* distally skewed subunits and is attached to the B-subfibers by a slender stalk. In the presence of ATP, this head shifts its orientation such that it can be seen to be constructed from two globular domains. One interpretation of these observations is that these structural changes represent distinct states of a cyclic cross-bridge cycle. The subfractionated samples of the outer-arm dynein were assessed by a translocation assay *in vitro*, in which putaitve motor protein was allowed to adsorb to a glass coverslip, and microtubules were then applied together with ATP. The "gliding" movement of microtubules under such conditions can be examined by video-enhanced contrast-differential interference contrast (VEC-DIC) microscopy [9]. This system was originally introduced to monitor the activity of microtubule-associated motor protein in cytosolic extracts of squid giant axons, with the resultant discovery of kinesin [10, 11]. The motor proteins, when properly oriented on a coverslip, can interact with a microtubule in such a way that they generate force along it, causing the microtubule to glide along the glass surface. Motors that are not properly oriented, rather than retarding the microtubule, seem unable to interact with it and have no apparent effect on the net production of force. Sale and Fox [12] observed that microtubules also glide on coverslips coated with just the Dβ HC/IC1 fraction. Neither the Dα HC nor IC2/IC3 fractions were associated with gliding of microtubules.

PROTEOLYTIC AND PHOTOLYTIC ANALYSES

The functional substructure (site of hydrolysis of ATP within the molecule) of Dβ HC was revealed by a classical approach rather than by molecular cloning. Ogawa [13] first obtained a tryptic fragment with ATPase activity from a low-salt extract of dynein and named it fragment A. Fragment A is a molecule of about 360–400 kDa in its native form and it can be separated into two peptides, designated f2 (190 kDa) and f3 (135 kDa), by SDS-PAGE [14]. Since f2 and f3 remain associated with each other during native PAGE, it is possible that the corresponding two regions of Dβ HC could be folded back on each other *via* intramolecular interactions. Ow *et al.* [15] established the principal pathway for tryptic cleavage of Dβ HC in a low-salt buffer, as shown in Figure 1. They isolated fragment B (also known as f1 peptide, 130 kDa) which is detached from fragment A during digestion. Dα HC did not generate a stable tryptic fragment. This result suggests that the two heavy chains that make up the outer arms are structurally

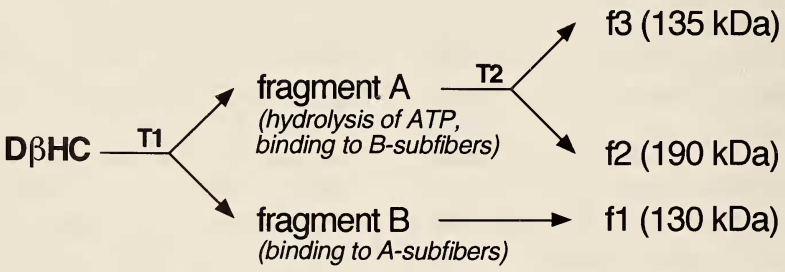

Fig. 1. Principal pathway of tryptic cleavage of Dβ HC. T1 indicates the early cleavage and T2 indicates the subsequent cleavage.

different from one another.

The outer-arm dynein has two microtubule-binding sites. The ability of isolated dynein to rebind to the extracted axonemes was reported by Ogawa and Mohri [16]. Functional recombination of isolated outer arms revealed that the outer arms bind to the A-subfibers in a salt-dependent manner [17]. This type of binding ability of the outer arms was not associated with fragment A [13]. Fragment B may contain the binding site for A-subfibers [15]. However, the ability of fragment B to rebind to the extracted axonemes has not yet been demonstrated. The outer arms can associate with the adjacent B-subfibers in an ATP-dependent manner, as described above [8]. Since fragment A has ATPase activity and is slightly activated by the B-subfiber fraction [13], outer-arm dynein may interact with the B-subfibers through the fragment A moiety of DβHC in a ATP-dependent manner.

Irradiation at 365 nm of DβHC in the presence of Mg-ATP and a low concentration of vanadate (V_i) cleaved DβHC at a single site termed the V1 site and ATPase activity decayed in a biphasic manner [18]. Because vanadate can potently suppress the activity of dynein ATPase, probably *via* occupation of a site that is normally reserved for the γ-phosphate of ATP, the V1 site probably lies in the hydrolytic domain of the DβHC. Irradiation in the presence of Mn^{2+} ions and of a higher concentration of V_i resulted in cleavage of DβHC at a single site, designated V2, but this cleavage at the V2 site was not correlated with any direct effect on ATPase activity [19]. The peptides produced by sequential cleavage at the V2 site and then the V1 site indicated that the two sites are separated by a region of 100 kDa along the length of the DβHC. The ATP-hydrolysis pocket of the central domain might be composed of the γ-P_i-binding V1 site and the purine-binding V2 site. The DβHC can be covalently modified by reaction with the hydrolyzable photoaffinity analog of ATP, 8-azido adenosine 5′-triphosphate (8-N_3ATP), which is hydrolyzed by fragment A at about 10% of the rate of hydrolysis of ATP [15]. The V2 site was found to be close to the locus of attachment of 8-N_3ATP, which may correspond to the purine-binding region of the ATP-hydrolytic site on the DβHC. Mocz *et al.* [20] proposed a map of the sites of tryptic and photolytic cleavage on the DβHC, as shown in Figure 2.

HUNTING FOR A GENUINE CLONE

A much more direct approach to the analysis of the functional site is provided by the molecular cloning of the gene for DβHC. Garber *et al.* [22] claimed initially that they had isolated cDNAs for the dynein heavy chain from trout testis that predicted an extensive, carboxy-terminal, α-helical coiled-coil domain. Because of incomplete characterization, it is unknown which of the several

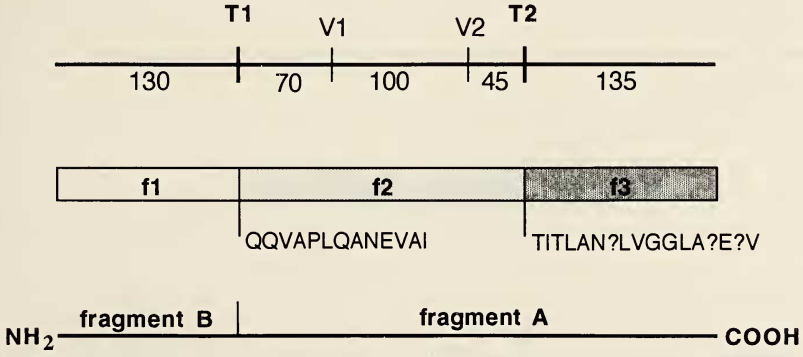

FIG. 2. Tryptic (T) and photolytic (V) sites within DβHC. The original map proposed by Mocz *et al.* [20] has been revised [21]. Numbers below the top map represent molecular masses in kDa, as determined by SDS-PAGE. The second map shows the positions of three tryptic fragments in the molecule. The amino-acid sequences of the f2 and f3 peptides are also shown. The bottom map shows that fragment A is located on the carboxy-terminal side of the molecule adjacent to fragment B.

heavy chains of trout dynein [23–25] these clones might encode. Mitchell [26] isolated genomic clones of α and β heavy chains from *Chlamydomonas*. However, none of these clones have yet been sequenced. Foltz and Asai [27] characterized a cDNA that encodes a portion of sea-urchin ciliary DβHC. Although four independent criteria suggest that their clone encodes a portion of DβHC, their identificaiton of immunoreactive clones from expression libraries cannot be taken as proof that the cDNA clone of interest has been isolated.

Hisanaga *et al.* [28] isolated "cytoplasmic dynein" with a molecular mass and immunogenicity similar to those of axonemal DβHC from unfertilized eggs of the sea urchin. The substructure [29] was also indistinguishable from that of the axonemal DβHC [8]. Ogawa *et al.* [30] showed that DβHCs from sperm and egg cilia may be similar to one another. There is no evidence to suggest that sea-urchin "cytoplasmic dynein" is different from ciliary or sperm DβHC. Recently, Ogawa [31] screened a cDNA library that corresponded to the poly(A)$^+$ RNA of unferilized eggs using an antibody directed against sperm axonemal dynein heavy chains. The cDNA clones (λJ292, λJ296, λA101, λA102, λA103, and λA104) obtained may encode ciliary DβHC. Fingerprints of fusion protein produced by lysogenic λJ296 were similar to those of authentic 21S dynein from sperm. The Northern blot of poly(A)$^+$ RNA revealed that only two clones (λJ296 and λA103) could hybridize with an RNA of ~16 kb in length. Since DβHC has an estimated mass of 480 kDa, it could be encoded by poly(A)$^+$ RNA of at least 14 kb in length. Thus, the two clones appear to be strong candidates. Finally, the amino-acid sequence deduced from the nucleotide sequence of λA103 contains one of the ATP-binding motifs (GKT site, see below) and the amino-terminal sequence of the f2 peptide [21]. Thus, these two clones appear to be the first genuine partial clones of cDNA that encodes DβHC.

CONSTRUCTION OF FULL-SIZE COMPLEMENTARY DNA

The λJ296 and λA103 clones encode the carboxy-terminal and central regions of DβHC, respectively. The missing segments of cDNA can be isolated by making mini cDNA libraries primed with oligonucleotides that are complementary to the 5′-portion of these clones, with subsequent screening with radiolabelled DNA probes. Ogawa [4] has sequenced additional three clones (λF1113, λA055, and λM062). Full-size cDNA was constructed by the overlapping of five clones, as shown in Figure 3. The long reading frame can encode a protein of 4,466 amino-acid residues with an unmodified molecular mass of 512 kDa. The deduced complete amino-acid sequence of DβHC

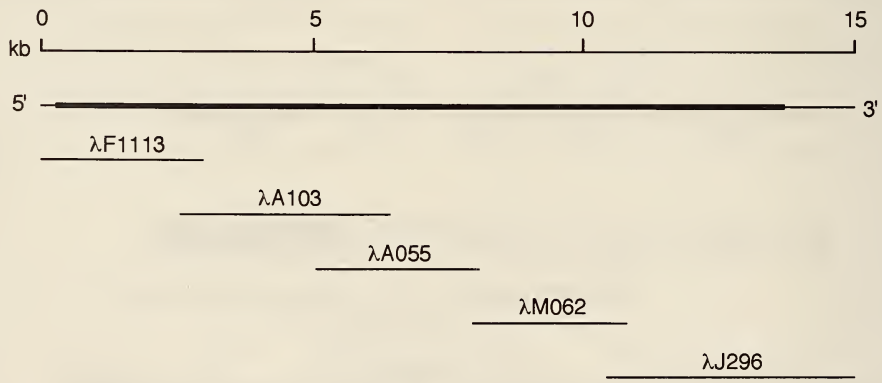

Fig. 3. Five overlapping clones that encode DβHC. The long open reading frame (thick line), flanked by non-conding sequences (thin lines), is shown at the top. Because of multiple allelic variation, the nucleotide sequences of two clones in the overlapping region are different from one another with frequency of one altered base per 100 bases. For overlapping of clones, weight was given to the nucleotide sequences of λJ296 in the case of λJ296 and λM062, λM062 in the case of λM062 and λA055, λA103 in the case of λA055 and λA103, and λA103 in the case of λA103 and λF1113.

is shown in Figure 4. The sequence was confirmed by the finding of the two amino-terminal sequences of the f2 and f3 peptides of fragment A in the deduced sequence. The former sequence was found at amino-acid residues, 1,192–1,204 and the latter at residues 3,324–3,340. The entire sequence shows no significant similarity to the partial sequences reported by Garber *et al.* [22] for the trout and by Foltz and Asai [27] for the sea urchin.

ATP-BINDING SITES

Fragment A corresponds to amino-acid residues 1,192–4,466. In view of the ability of fragment A to hydrolyze ATP, a search was made in this region for the consensus ATP-binding motif

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1     MGDVVDARLD FISEYILKSY KLKPDKWTKC INVEENKILM LEFLEKADNP QLVFTVNPAG LITPSYEFPS ALKNTKAIYF IKKGREPVGK
91    DNIKTNLVYG DLSYTPLEQL SALVDEVLVP LLANPRNHEQ WPVVVSQDVL RHVHNLKSSV YVVAGQVKGK TLLPLPVGSE KVETAAGSEE
181   KDDSYDRSLV HAIESVIIDW THQIRDVLKR DSAQPLLEGL NPGPMVEINF WKAKCENLDC IFQQLRDPKV RKMKELLERT QSSYLPSFNN
271   IERDVEAALT EAQDINIHLK PLVYQIESMD ELEFSDLTPR LAPILHTVCL IWSNSDYYNT APRVIVLLQE ICNLLIDLCR TFLDPSEIFK
361   LEPEESLEKV RGALTVLKNW RELYDEHRAK LKDYFKDGKE VKEWEFASPL VFTRMDNFIR RIETIQSLFE TNVEFSKLEK TEMGSMKGRM
451   LSQQVEKIHE EFQECAKVFT ERPYDGLDPT CQEFLEDYEE FEKKVFDLDR RLGSILCQGF DDCCGLEAAF KMLDCYGPLL DRPVIRNDFE
541   CKYPIVLMLY DQELDQSKEI YDEHMRVEEA NGNAPLNKNM PDVAGQLKWS AQLRDRISKP MGSLKHMEHP TGVRRILESE DAKVIFQKYE
631   EMLNLLNKYE QKVFENWTKG VDEVCKTNLD QSLITRDDAS KLIMVNFDPK LVSVLREVKY LQIRGEETIP ESAASIYEKH ETLRKYVANL
721   DLTVAWYNKV RKTVLEVEFP LIEGQLADLD TRLRQAEADL NWTSDSVWEY IQETRDQVRD LEKRVQQTKD NVDRIKKIMA EWTKQPLFER
811   KELKKESLLA LDDRQDRLKK RYAEITTAGE KIHSLMKENL DLFKAEASSD IWKAYVDYVD DMVIDGFFNC IHCTLTYLLE NTDPRHCAAP
901   LFEARLELQV PDMIFNPSLD YGIADGFYDL VEMLISDTYK MASLVNRLAE HNGQEHYQAD LEGMDDLSDV RNDLMDRVQT IMTKAQEYRN
991   SFDNYAYLYV DDRKEFMRQF LLYNHVLTTE EIEAHAEDGV PECPPTLDQF KEQVDTYEKI YSEADEIEPE QVFDAWFRVD SKPFKAALLN
1081  IIKKWSFMFK QHLIDHVTNS LSELQEFIKV GNSGLTKTVE DGDYNGLVDC MGHLMAVKER QAATDEMFEP IKQTIELLKT YDQEMSEEVH
1171  TQLQELPEQW NNTKKIAITI KQQVAPLQAN EVAIIRRKCT SFDVRQHEFR ERFRKEAPFI FLFDGPYQCL DKCHSEIYEM EEHMAKLQES
1261  AGLFEVNMPD YKQLKACRRE VRLLKGLWDL IMVVRTSIED WKTTPWLEIN VEQMEMDCKK FAKDIRSLDK EMRAWDAYNG LDATVKNMLT
1351  SLRAVSELQN PAIRERHWQQ LMAATKVKFT MDKETTLSDL LALNLHNFED EVRNIVDKAV KEMGMEKVLK ELNTTWSSMD FDYEPHSRTG
1441  ISLLKSNEEL IETLEDNQVQ LQNLMTSKHI AHFLEEVSGW QKKLSTTDSV ITIWFEVQRT WSHLESIFIG SEDIRNQLPE DSKRFDGIDT
1531  DFKELAAEME KTPNVVEATN KARLFDRLEA IQGSLVVCEK ALAEYLETKR LAFPRFYFVS SADLLDILSQ GNNPTQVQRH LSKLFDNMAK
1621  LKFKQDDEGN DTKLALGMYS KEGEYVDFDK ECECTGQVEV WLNRVMDTMR STVRSQFADA VVSYEEKPRE QWLYDYPAQV ALATTQVWWT
1711  TEVNISFARL EEGHENSMKD YNKKQILQLN TLIGLLIGKL TKGDRQKIMT ICTIDVHARD VVAMMVLKKV DSAQAFQWLS QLRHRWADDD
1801  KHCYANICDA QFKYSYEYLG NTPRLVITPL TDRCYITLTQ SLHLVMSGAP AGPAGTGKTE TTKDLGRALG IMVYVFNCSE QMDYKSCGNI
1891  YKGLAQTGAW GCFDEFNRIS VEVLSVVAVQ VKCVQDAIRD KKERFNFMGE EISLIPSVGI FITMNPGYAG RTELPENLKA LFRPCAMVVP
1981  DFELICEIML VAEGFLEARL LARKFITLYT LCKELLSKQD HYDWGLRAIK SVLVVAGSLK RGDPQRPEDQ VLMRALRDFN VPKIVSDDTP
2071  VFMGLIGDLF PALDVPRRRD LDFEKVVKQS TLDLKLQAED SFVLKVVQLE ELLAVRHSVF VIGNAGTGKS QVLKVLNKTY SNMKRKPVFI
2161  DLNPKAVTND ELFGIINPAT REWKDGLFSV IMRDMSNITH DGPKWIVLDG DIDPMWIESL NTVMDDNKVL TLASNERIPL TPSMRLLFEI
2251  SHLKTATPAT VSRAGILYIN PSDLGWNPIV TSWIDTREVQ SERANLTILF DKYLPTLLDT LRIRFKKIIP IPEQSMVQML CYLLECLLTP
2341  ENTPADCPKE LYELYFVFAS IWAFGGSMFQ DQLVDYRVEF SKWWITEFKT IKFPNQGTVF DYYIDQESKK FLPWSEKVPT FELDPEIPMQ
2431  AVLVHTNETT RVRFFMDLLM ERGRPVMLVG NAGLGKSVLV GDKLSNLGED SMVANVPFNY YTTSEMLQRV LEKPLEKKAG RNYGPPGTKK
2521  LVYFIDDMNM PEVDTYGTVQ PHTLIRQHMD YKHWYDRAKL TLKEIHKCQY VSCMNPTSGS FTINSRLQRH FCVFALSFPG QDALSTIYNS
2611  ILSQHLANIA VSNALQKLSP TVVSATLDLH KKVAQSFLPT AIKFHYVFNL RDLSNVFQGL LYSGSDLLKS PIDFARLWMH ECQRVYGDKM
2701  INDQDIEAFE KLVFEYAKKF FEDVDEEALK AKPNIHCHFA TGIGDPKYMP CATWPELNKI LVEALDTYNE INAVMNLVLF EDAMQHVCRI
2791  NRILESPRGN ALLVGVGGSG KQSLARLASY ISSLEVFQIT LRKGYGIPDL KLDLATVCMK AGLKNIGTVF LMTDAQVSDE KFLVLINDLL
2881  ASGEIPDLFA DDEVENIIGG VRNEVKGMGL QDTRENCWKF FIDRLRRQLK TVLCFSPVGT TLRVRSRKFP AVVNCTSIDW FHEWPQEALV
2971  SVSKRFLDEV ELLKGDIKNS IAEFMAYVHV SVNESSKQYL TNERRYNYTT PKSFLEQIKL YESLLAMKSK ELTAKMERLE NGLTKLQSTA
3061  QQVDDLKAKL ASQEVELAQK NEDADKLIQV VGVETEKVSK EKATVDDEEK KVAIINEEVS KKAKDCSEDL AKAEPALLAA QEALNTLNKN
3151  NLTELKSFGS PPSAVLKVAA AVMVLLAPNG KIPKDRSWKA AKVVMNKVDA FLDSLINYDE ENIHENCQKA IKEYLNDPEF EPEYIKGKSL
3241  AAGGLCSWVV NIVKFYNVYC DVEPKRIALQ KANDELKAAQ DKLALIKAKI AELDANLAEL TAQFEKATSD KLKCQQEAEA TSRTITLANR
3331  LVGGLASENV RWGEAVANFK IQEKTLPGDV LLITAFVSYI GCFTKNYRVD LQDRMWLPFL KSQKDPIPIT EGLDVLSMLT DDADIAVWNN
3421  EGLPSDRMST ENATILSNCQ RWPLMIDPQL QGIKWIKQKY GDELRVIRIG QRGYLDTIEN AISSGDTVLI ENMEESIDPV LDPVLGRNTI
3511  KKGRYIKIGD KEVEYNPEFR LILQTKLANP HYKPEMQAQT TLINFTVTRD GLEDQLLANV VAQERPDLEK LKSDLTKQQN DFKIILKELE
3601  DNLLSRLSSA EGNFLGDTAL VENLETTKRT AAEISVKVEE AKVTEVKINE ARELYRPAAA RASLLYFILN DLNKINPIYQ FSLKAFNTVF
3691  SLSIARAEPC EDVKERVVNL IDCITYSVFI YTTRGLFEAD KLIFTTQVAF QVLLMKKEIA QNELDFLLRF PIQVGLTSPV DFLTNSAWGA
3781  IKSLSAMEDF RNLDRDIEGS AKRWKKFVES ECPEKEKFPQ EWKNKSALQK LCMMRALRAD RMSYAVRNFI EEKLGSKYVE GRQVEFAKSY
3871  EETDPATPVF FILSPGVDPL KDVEALGKKL GFTFDNNNFH NVSLGQGQEI VAEQCMDLAA KEGHWVILQN IHLVAKWLST LEKKLEQYSI
3961  GSHESYRVYM SAEPAGSPES HIIPQGILES SIKITNEPPT GMFANLHKAL YNFNQDTLEM CAREAEFKVI LFALCYFHAV VCERQKFGPQ
4051  GWNRSYPFNT GDLTISVNVL YNYLEANSKV PWQDLRYLFG EIMYGGHITD DWDRRLCRTY LEEYMAPEML DGDLYLAPGF PVPPNSDYKG
4141  YHQYIDEILP PESPYLYGLH PNAEIGFLTT ESDNLFKVVL ELQPRDAGGG GGGGSSREEK IKSLLDEIVE KLPEEFNMME IMGKVEDRTP
4231  YVVVAFQECE RMNTLTSEIR RSLKELDLGL KGELTITPDM EDLSNALFLD QIPASWVKRA YPSLFGLSAW YADLLQRIKE LEQWTADFAL
4321  PNVVWLGGFF NPQSFLTAIM QSMARKNEWP LDKMCLQCDV TKKNKEDFSS APREGSYVHG LFMEGARWDT QTNMIADARL KELAPNMPVI
4411  FIKAIPVDKQ DTRNIYECPV YKTKQRGPTF VWTFNLKSKE KAAKWTLAGV ALLLQV
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FIG. 4. Deduced amino-acid sequence of sea-urchin *Anthocidaris crassispina* axonemal DβHC. The amino-acid residues that have been confirmed by direct sequencing are underlined and putative-ATP-binding motifs are indicated by filled circles. Amino acids marked with filled squares define the sites of trypsin cleavage.

GXXXXGK(T/S), where X is any amino acid. This motif was found at three positions in the f2 peptide as follows, between residues 1,192 to 3,323: beginning at Gly 1,852 (termed the GKT site); at Gly 2,133 (the GKS1 site); and at Gly 2,460 (the GKS2 site). The sequence GXXXSGK is also accepted as an ATP-binding sequence of adenylate kinases [32], and this sequence was also found in the f2 region, beginning at Gly 2,805 (the SGK site). Therefore, molecular cloning of DβHC has revealed the presence of four putative ATP-binding sites in the middle region of the molecule.

Since the amino-terminal sequence of the f2 peptide begins at residue 1,192, the T1 site can be identified on the carboxy side of Lys 1,191. According to the map of DβHC (Fig. 2), the γ-P_i-binding site (V1 site) is separated by a region of 70 kDa from the T1 site in the carboxy-terminal direction. There are 660 amino-acid residues between Gln 1,192 of the T1 site and Gly 1,852 of the GKT site, and this distance is equivalent to a peptide of 73 kDa. Thus, the GKT site corresponds to the V1 site revealed by photocleavage of DβHC and may be able to catalyze the hydrolysis of ATP. The binding site for 8-N_3ATP (V2 site) is separated by a region of 170 kDa from the T1 site in the carboxy-terminal direction. There are 1,603 amino-acid residues between Gln 1,192 of the T1 site and Gly 2,805 of the SGK site, and this distance is equivalent to a peptide of 177 kDa. Thus, the SGK site corresponds to the V2 site. Since fragment A can hydrolyze 8-N_3ATP at about 10% of the rate of hydrolysis of ATP, the SGK site may also be able to catalyze the hydrolysis of ATP. The presence of two GKS sites was not predicted by the photocleavage of DβHC. The sites have sequences that are very similar to one another. The ATP-dependent *ClpA* protease of *E. coli* has also two ATP-binding motifs which are very similar to one another [33]. Thus, the sequence similarity between the two GKS sites in the molecule may not be a coincidence, but may represent proof of two functional sites for hydrolysis of ATP. Fragment B does not have any ATPase activity [15]. The sequence AXXXXGKT, beginning at Ala 154, appears to be a modified nucleotide-binding motif, as found in the GTPase superfamily [34]. At the present time, however, it is uncertain whether fragment B has the ability to bind GTP and catalyze its hydrolysis.

The position of the ATP-binding motif on a motor molecule may be related to the directionality of movement along a microtubule. Both dynein and kinesin are microtubule-motor proteins and they move in opposite directions along a microtubule. The striking difference between the amino-acid sequences of both motor proteins is reflected in differences between the positions of the ATP-binding motifs on their heavy chains; the motif is located at the amino terminus of the kinesin heavy chain [35] and in the midregion of DβHC. The product of the *claret* (or *ncd*) gene belongs to the kinesin superfamily. It is noteworthy that the ATP-binding motif is located at the midregion of this gene product [36, 37] and the molecule moves toward the microtubule's minus end [38, 39], a direction characteristic of dynein [40].

POLYMORPHISM

The nucleotide sequence shows two types of polymorphism (Fig. 5). When λF1113 was isolated, 14 additional shorter cDNAs were also obtained. Two clones, λF1106 and λF1114, were sequenced and their cDNAs overlapped the sequence of λF1113. Fifteen bases common to both λF1106 and λF1113, which encode five amino acids, were absent in the sequence of λF1114 (possibly as a result of alternative splicing). Furthermore, the underlined nucleotide sequence in

Residue number	*609*	*610*	*611*	*612*	*613*	*614*	*615*	*616*	*617*
	H	P	T	G	V	R	R	I	L
λF1113	CAT	CCG	ACC	GGT	GTC	AGG	AGA	ATT	TTG
λF1106	CAT	CCG	ACC	GGT	GTC	AGG	AGG	ATT	TTG
λF1114	CAT	CCG	---	---	---	---	---	ATT	TTG

Fig. 5. Polymorphism of cDNA clones that encode DβHC.

Figure 5 differed between λF1106 and λF1113 (possibly as a result of multiple alleles). The latter type of polymorphism occurs at a rate of about one base per 100 bases in the two overlapping clones but, so far, no substitutions of amino acids have been found. Since the full-size cDNA was constructed by overlapping of the present five clones, which include λF1113, the amino-acid sequence of Dβ HC described here is just one possible sequence, and slightly longer and shorter versions may also be present in the sea urchin.

SECONDARY STRUCTURE

The secondary structure of Dβ HC was analyzed by Dr. Ken Nishikawa of the Protein Engineering Research Institute, Osaka, Japan (Fig. 6). There are two long α-helix-dominant regions (termed α1 and α2) in the sequence, suggesting that the Dβ HC is composed of three large β-structure-dominant domains (termed the N, M, and C domains) separated by these regions. The M domain is split by short α-helix-dominant regions into four smaller β-structure-dominant regions, and the C domain is similarly split into three smaller region. Although analysis of secondary structure predicts that the α1 region is rich in α helix, there are no long hydrophobic heptad repeats without interruption, as are found in the α-helical coiled-coil regions of filamentous motor proteins such as myosin and kinesin. The α2 region contains two heptad repeats, which are predicted to be largely α-helical, at amino-acid residues 3,028–3,153 and 3,234–3,338 with interruptions, as shown in Figure 7. In particular, two leucine heptad repeats at residues 3,028–3,083 and 3,262–3,303 could favour the formation of a leucine zipper structure, with resultant generation of a large globular structure from the M and C domains. This leucine zipper structure may explain why f2 and f3 peptides remained together in fragment A during tryptic digestion of Dβ HC, while f1 was detached, as described above.

MODEL OF THE STRUCTURE

Figure 8 shows a model of structure of Dβ HC, as deduced from the predictions about secondary structure and the proteolytic analysis of the authentic protein. Quick-freze deep-etch electron microscopy of the Dβ HC/IC1 complex revealed that the complex is composed of a pear-shaped head and an irregularly shaped stem, while the base looks like a small globular bead [8]. According to this structural model, the N domain may correspond to the base, the α1 region to the irregularly shaped stem, and the associated M and

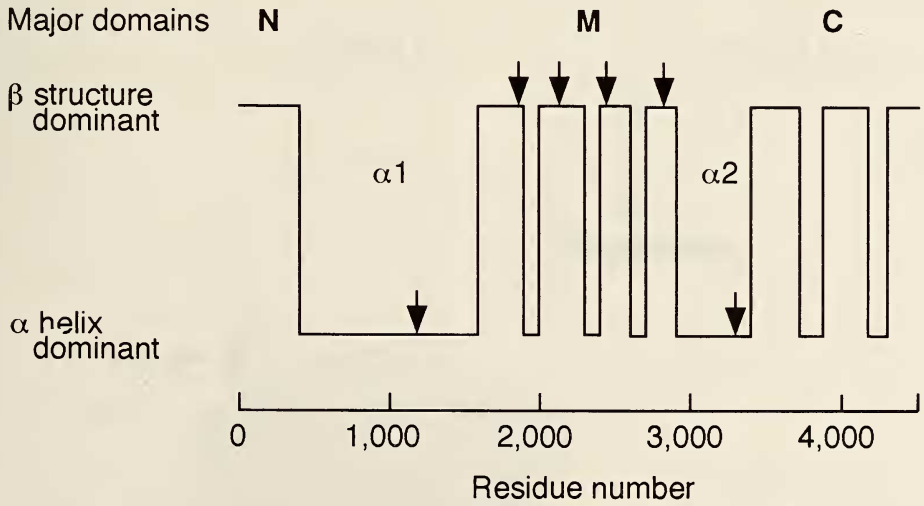

FIG. 6. Predicted secondary structure of the Dβ HC. The four arrowheads in the M domain indicate the GKT, GKS1, GKS2, and SGK sites of the ATP-binding motifs, from the left. The arrowheads in the α1 and α2 regions indicate the T1 and T2 sites, respectively.

C domains to the pear-shaped head. As described above, it has also been documented that the outer arm *in situ* attaches to the B-subfibers of adjacent outer-doublet microtubules *via* a slender stalk. This slender stalk is seen neither in the isolated outer arms nor in the DβHC/IC1 complex. It is possible that the slender stalk corresponds to the α2 region of the present structural model.

A NEW FAMILY OF MICROTUBULE-BINDING MOTOR PROTEINS

As described above, DβHC appears to have two types of microtubule-binding site in the molecule. It may form a stable complex with the A-subfibers of axonemal doublet microtubules in the fragment B region. MAP2 [41] and tau [42] proteins form stable complexes with microtubules; they have regions of homologous sequence at their carboxy termini, where there are three 18-residue repeats

	d	e	f	g	a	b	c	d	e	f	g	a	b	c	d	e	f	g	a	b	c
3,028	**I**	K	L	Y	E	S	L	**L**	A	M	K	S	K	E	**L**	T	A	K	**M**	E	R
3,049	**L**	E	N	G	**L**	T	K	**L**	Q	S	T	**A**	Q	Q	**V**	D	D	L	**K**	A	K
3,070	**L**	A	S	Q	E	V	E	**L**	A	Q	K	N	E	D	**A**	D	K	L	**I**	Q	V
3,091	**V**	G	V	E	**T**	E	K	**V**	S	K	E	**K**	A	T	**V**	D	D	E	E	K	K
3,112	**V**	A	I	I	N	E	E	**V**	S	K	K	**A**	K	D	**C**	S	E	D	**L**	A	K
3,133	**A**	E	P	A	**L**	L	A	**A**	Q	E	A	**L**	N	T	**L**	N	K	N	N	L	T
3,234	**Y**	I	K	G	**K**	S	L	**A**	A	G	G	**L**	C	S	**W**	V	V	N	**I**	V	K
3,255	**F**	Y	N	V	**Y**	C	D	**V**	E	P	K	R	I	A	**L**	Q	K	A	N	D	E
3,276	**L**	K	A	A	Q	D	K	**L**	A	L	I	**K**	A	K	**I**	A	E	L	D	A	N
3,297	**L**	A	E	L	**T**	A	Q	**F**	E	K	A	**T**	S	D	**K**	L	K	C	Q	Q	E
3,318	**A**	E	A	T	S	R	T	**I**	T	L	A	N	R	L	**V**	G	G	L	**A**	S	E

FIG. 7. Hydrophobic heptapeptide repeats in the α2 region. The numbers on the left indicate the residue number from the deduced amino-acid sequence of DβHC. There is a disruption of the repeats from Glu 3,154 to Glu3233. Bold letters indicate hydrophobic amino acids.

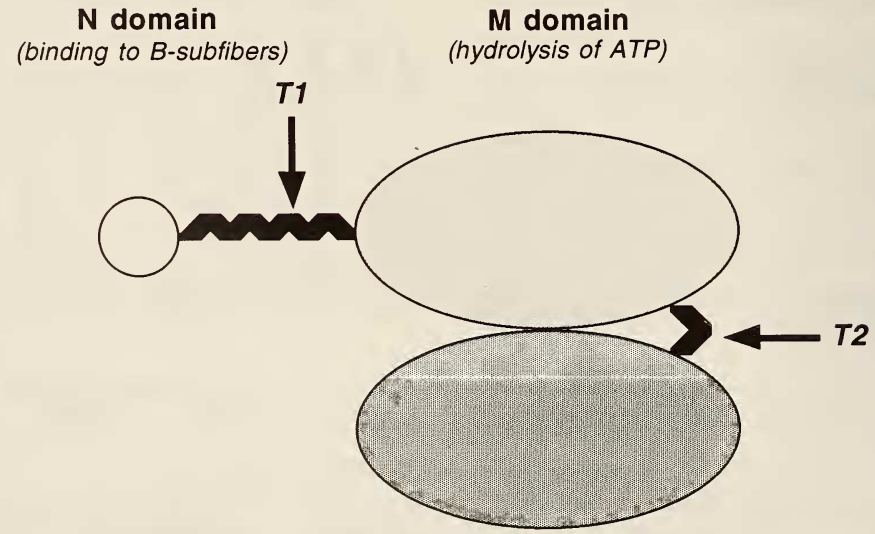

FIG. 8. A model of the structure of DβHC. T1 and T2 indicate the principal sites of trypsin cleavage.

that have been proposed as the microtubule-binding site. These repeated sequences are not found in the N domain of the DβHC sequence. DβHC transiently associates with the B-subfibers of the adjacent doublet microtubules during the ATP-hydrolytic cycle. Members of the kinesin superfamily of microtubule motor proteins also associate transiently with microtubules during the ATP-hydrolytic cycle. They share a region of sequence homology that extends from the ATP-binding site towards the carboxy-terminal end of the molecule, and this sequence has been suggested to constitute the site of ATP-dependent binding to microtubules [35]. This sequence homology is not found in the C domain, which extends from the M domain (multiple ATP-binding sites) toward the carboxy-terminal end of the molecule, or anywhere else in the amino-acid sequence of DβHC. Thus, DβHC seems to be a member of a new family of microtubule-binding motor proteins with unique microtubule-binding sequences that are unlike those of MAP2, tau, or members of the kinesin superfamily.

DYNEIN SUPERFAMILY

It is natural to speculate that dynein may also be involved in motile functions associated with cytoplasmic microtubules. Immunologocal studies, using antibodies directed against axonemad dyneins, have shown that the segregation of chromosomes during mitosis [43–47] and meiosis [48], and the translocation of melanophore in fish [49] could involve dynein motor molecules. MAP1C is a member of a class of five extremely high-molecular-weight microtubule-associated proteins that co-purify with brain microtubules [50] and it is responsible for retrograde transport [51], a property of axonemal dynein. MAP1C has been now found to be a cytoplasmic form of ciliary and flagellar dynein [52]. Since microtubules have been shown to be responsible for the transport of membranous organelles within the cytoplasm and, thereby, to play a role in axonal transport, secretion, endocytosis and transcytosis, cytoplasmic dynein could have very general functional role in cells. Isolation of a clone for cytoplasmic dynein is now the goal of many gorups.

Axonemal and cytoplasmic dyneins may constitute a superfamily of force-generating proteins, with each member possessing a conserved force-generating domain joined to a different "tail" that confers specific attachment properties. The outer- and inner-arm dyneins attach to different sites on axonemal doublet microtubules, while cytoplasmic dyneins interact with organelles and chromosomes. Structural and enzymatic studies suggest that the motor domains of the dyneins are similar to one another [see ref. 53 for a review]. Therefore, it is likely that the members of the dynein superfamily share a common motor domain that is linked to a distinct tail with unique binding properties in each case.

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